Towards Affordable Conjugate Vaccines

Efficient Chemistry and Low Cost CRM_{197} Carrier Protein

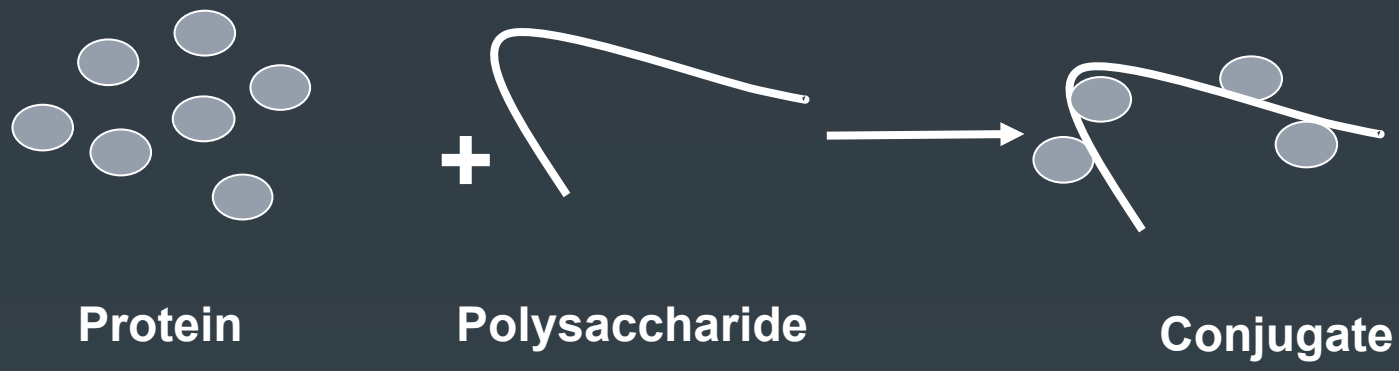
Andrew Lees

EuroVaccines
Rome, Italy
June, 2016



Conjugate Vaccines

Covalent linkage of carbohydrates to proteins to convert a T independent antigen into a T dependent antigen

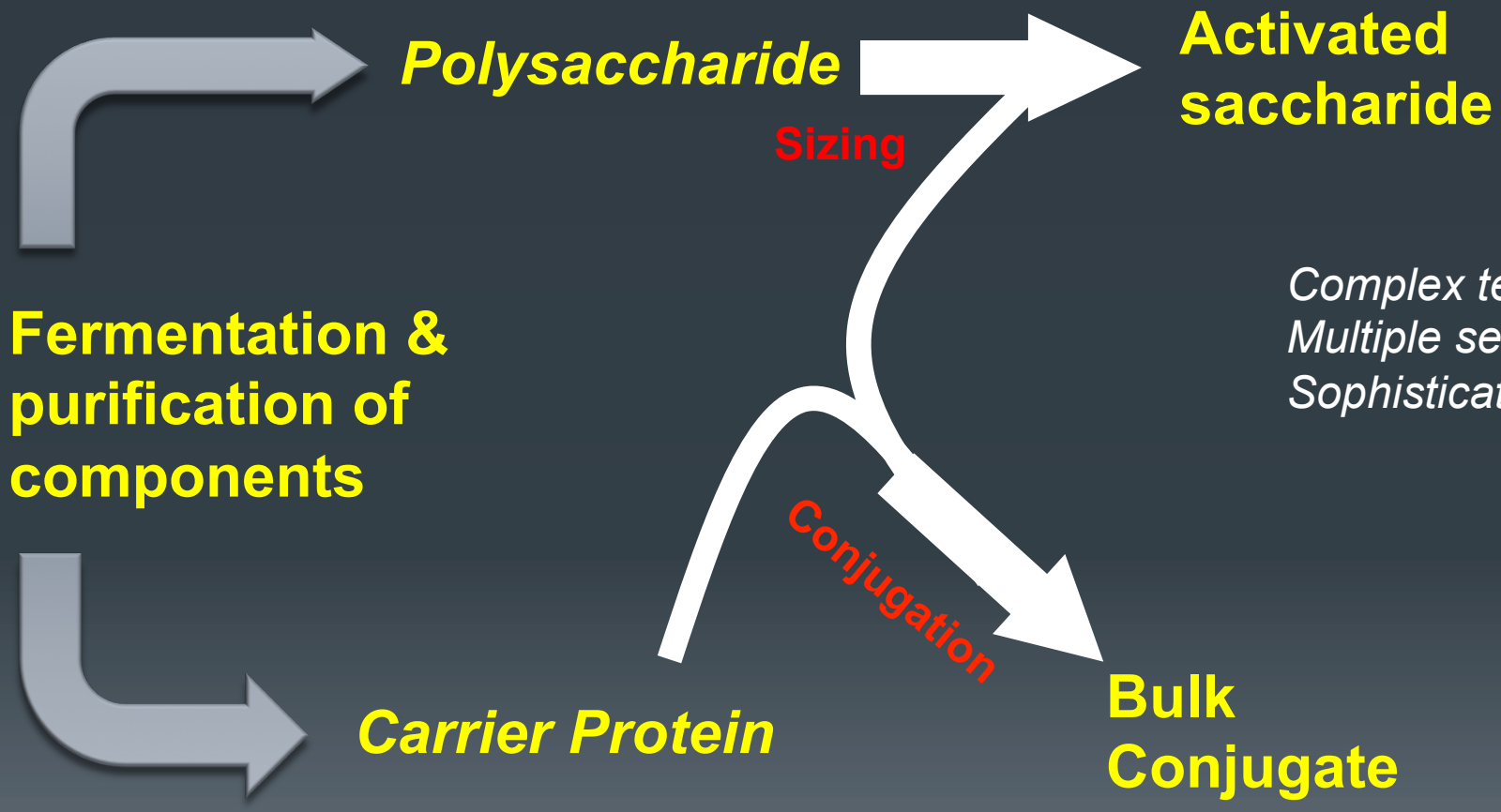


Currently licensed (US)

Streptococcus pneumoniae (Prevnar, Synflorix)
Haemophilus influenzae
Neisseria meningitidis

- ***Class switching***
- ***Boost (memory)***
- ***Affinity maturation***
- ***Immunogenic in infants***

Conjugate Vaccine
Process Flow



Conjugate Vaccines are Effective

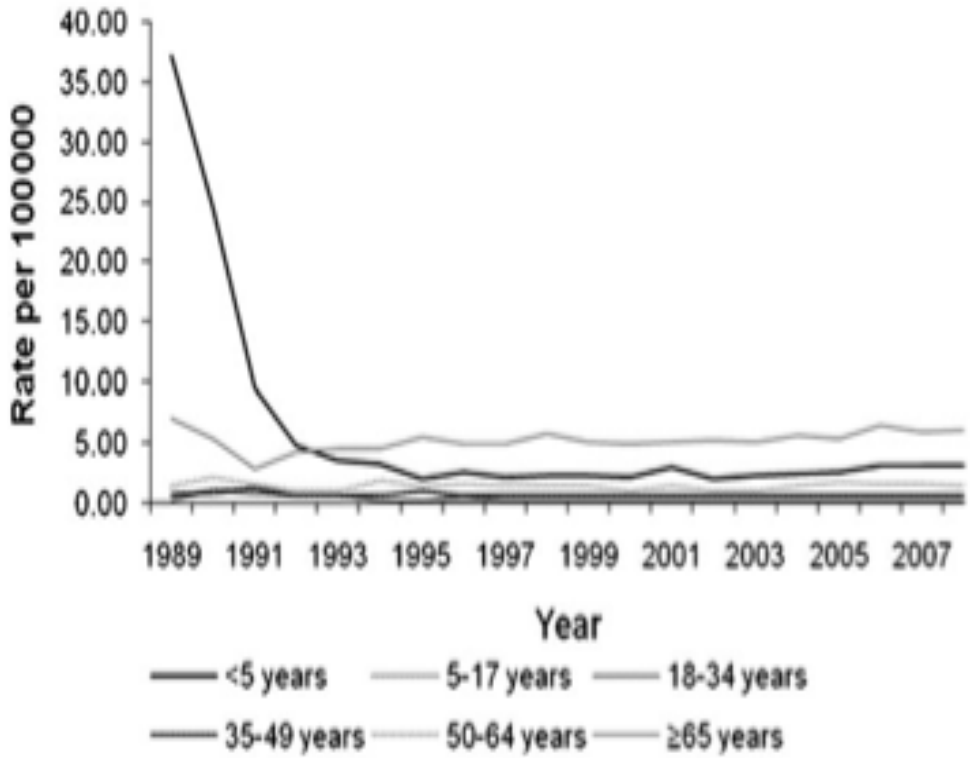


Figure 1. Trends in incidence of invasive *Haemophilus influenzae* disease, by age group—United States, 1989–2008.

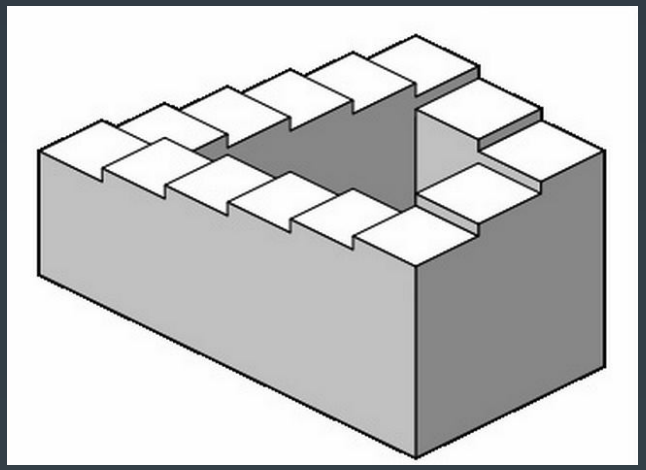
Epidemiology of Invasive *Haemophilus influenzae* • CID 2011:53

Conjugate Vaccine Costs

Pricing model

Supply

Component costs
- Carrier protein
- Polysaccharide



Dosing
- Dose
- Schedule

Conjugation
- Complexity
- Yield

Intellectual property

Storage
- Cold chain
- Thermal stable

Development

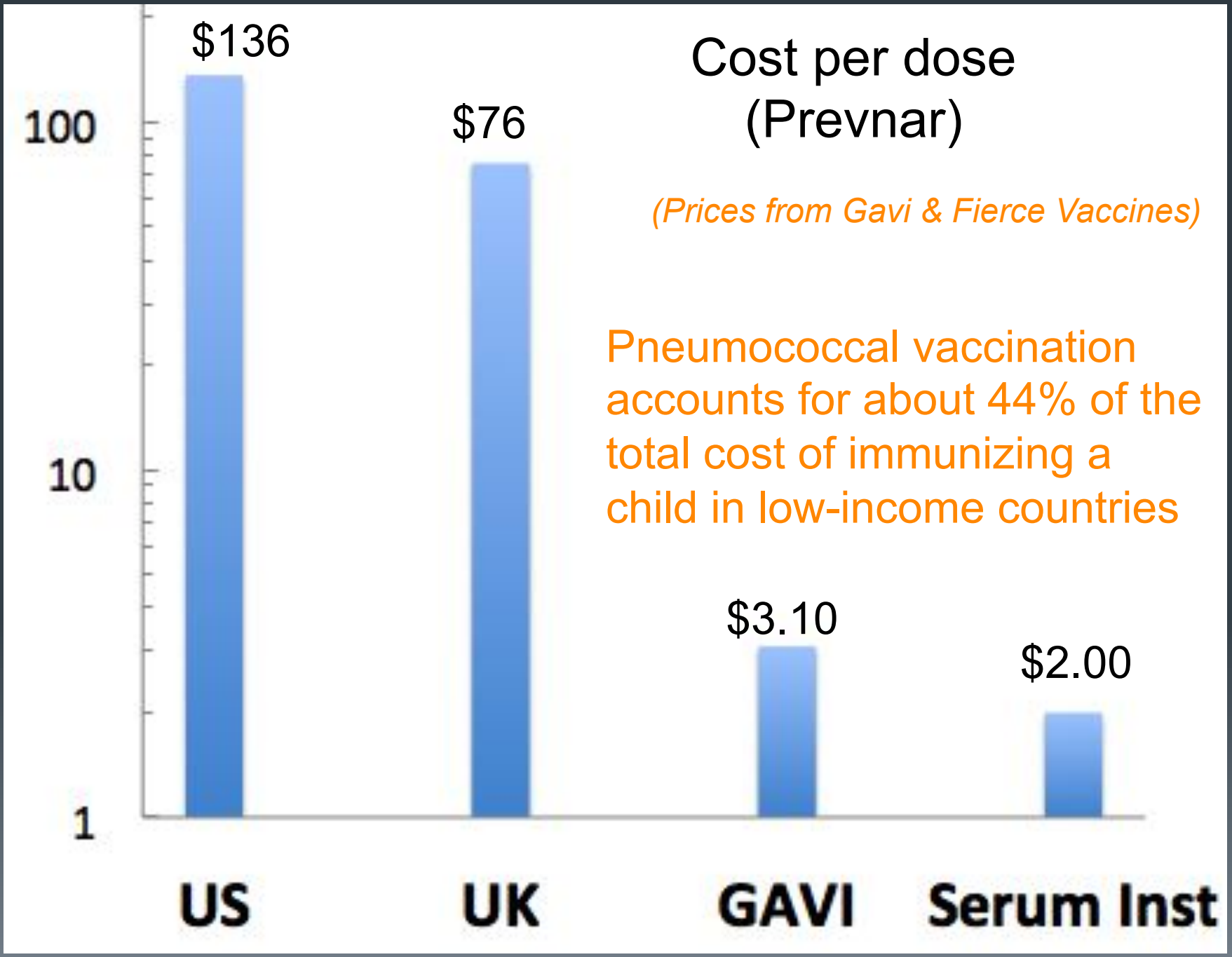
Regulatory compliance

Facilities
Labor

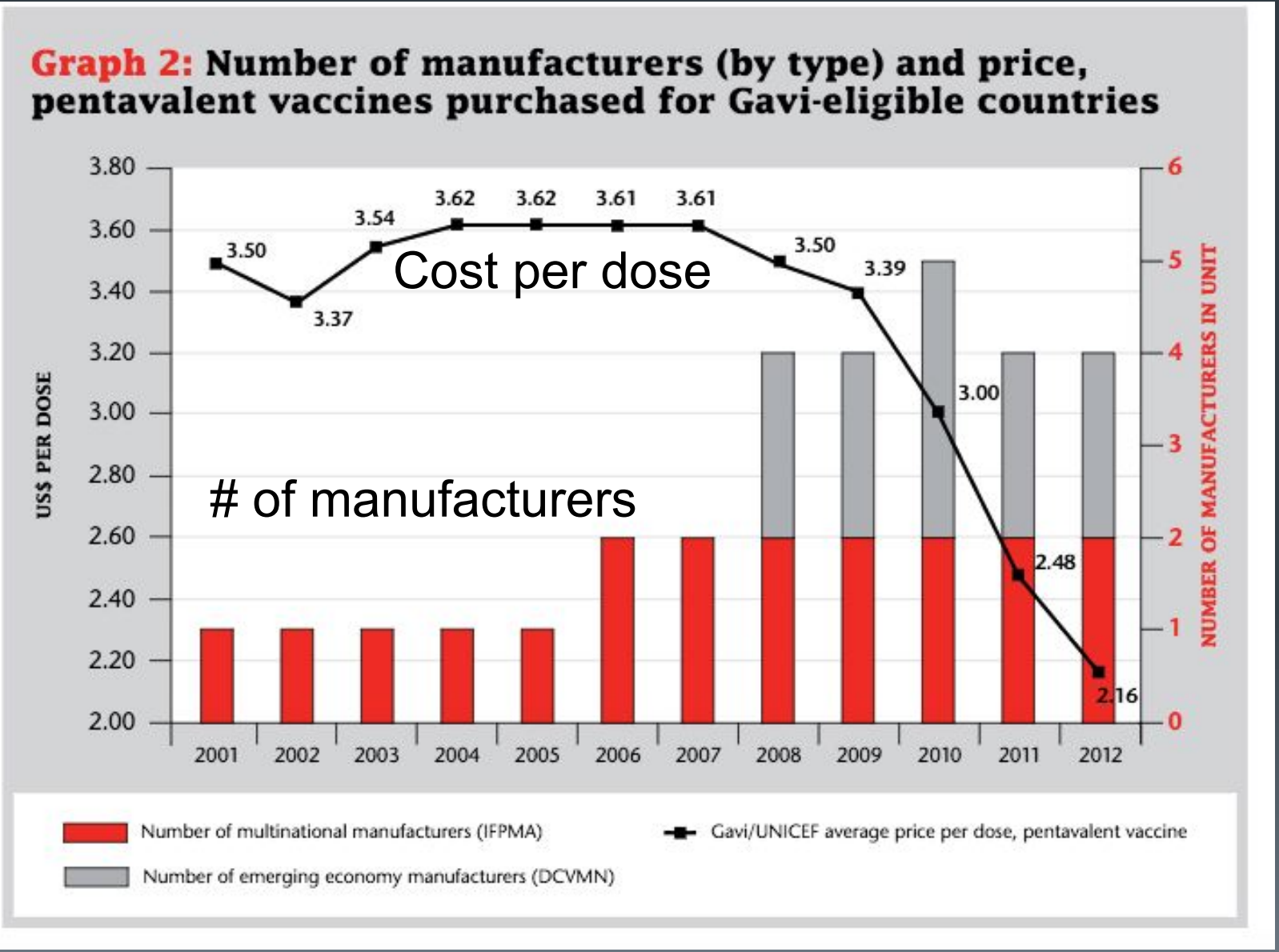
Fill & Finish

Vial

Formulation costs
- Adjuvant
- lyophylization


Cost per dose
(Prevnar)
(Prices from Gavi & Fierce Vaccines)
Pneumococcal vaccination accounts for about 44% of the total cost of immunizing a child in low-income countries
$136
$76
$3.10
$2.00
100
10
1
US
UK
GAVI
Serum Inst

Competition drives down price



Intellectual Property

Confidential

Chemistry patents

Merck patent effectively blocked use of thio-ether chemistry

Composition & formulation patents

Certain combination of serotypes, proteins, chemistry

Medecins Sans Frontieres files to block Pfizer patent on pneumonia vaccine in India

If India granted Pfizer a patent on its Prevnar 13 pneumonia vaccine, Indian firms would not be able to produce affordable versions of it for domestic use or exports, MSF said.

Affordable Conjugate Vaccines

- *More Price Competition*
  - *Increase number of suppliers*
    - *Capability*
      - *knowledge, training, expertise*
  - *Increase Capacity*

- *A more appropriate business model*
  - *Cost plus vs "value"/tiered pricing*

- *Four Reasons Drugs Are Expensive, Of Which Two Are False*
  - Jack Scannell, Forbes online 10 13 15

http://www.forbes.com/sites/matthewherper/2015/10/13/four-reasons-drugs-are-expensive-of-which-two-are-false/

COST of CONJUGATE VACCINE COMPONENTS

Polysaccharide

Protein

COST Of CONJUGATE VACCINE COMPONENTS

Polysaccharide production
Goal >500 mg/L

- *Strain development*
  - Screening of strains, polysaccharide identity and preliminary characterization
  - Research cell banks
- *Process optimization*
  - Fermentation: fed-batch process development
  - Purification optimization
  - In-process characterization
- *Process scale-up*
  - 50-100L scale

COST of CONJUGATE VACCINE COMPONENTS

Polysaccharide

Protein

Carrier Proteins

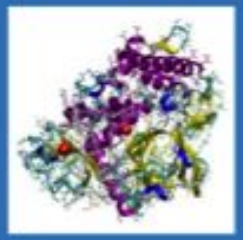
Tetanus Toxoid (TT)	Diphtheria Toxoid (DT)	Cross-Reactive Material 197 (CRM_{197})	*N. meningitidis* Outer Membrane Protein (OMP)	Non-Typeable *H. influenzae* Derived Protein D (PD)
				
• Derived from *Clostridium tetani* • Inactivated with formalin • Purified with ammonium sulfate and filter sterilized prior to conjugation process	• Derived from *Corynebacterium diphtheriae* • Detoxified with formaldehyde • Purified by ammonium sulfate fractionation and diafiltration	• Enzymatically inactive, nontoxic mutant of diphtheria toxin • Requires no formaldehyde detoxification • Obtained at near 100% purity	• Outer membrane protein complex derived from *N. meningitidis* serogroup B strain 11 • Purified by detergent extraction, ultracentrifugation, diafiltration, and sterile filtration	• Antigenically conserved surface lipoprotein found in all *H. influenzae* • Used in a nonacylated, antigenically active form
• 140 kD	• 63 kD	• 63 kD	• 37 kD	• 42 kD

Fig. 1. Carrier proteins used in *Haemophilus influenzae* b, *Neisseria meningitidis*, and *Streptococcus pneumoniae* conjugate vaccines [2,5–8].

Please cite this article in press as: Knuf M, et al. Comparative effects of carrier proteins on vaccine-induced immune response. Vaccine (2011), doi:10.1016/j.vaccine.2011.04.053

CRM_{197}: *preferred carrier protein*

- Used in Pfizer's Prevnar, the "gold standard"
- Less interference between serotypes than with TT
- Reduced carrier induced epitope suppression
- Pneumo vaccines using other carriers have not shown non-inferiority to Prevnar for all serotypes.

CRM and Conjugate Vaccines

Vaccine	CRM_{197}	PS
Prevnar13	**34 µg**	51 µg
Menveo	**50 µg**	25 µg
HibTiter	**25 µg**	10 µg

Current Sources of CRM_{197}

- *Corynebacterium diphtheriae ("native CRM")*
  - Low yield & difficult to grow
  - 50-100 mg/L
- *Pseudomonas fluorescens (Pfenex)*
  - Expensive
  - Not available for license in India or China
- *In Development*
  - Refolding from inclusion bodies
  - Low yield
  - Difficult process

Development of low cost CRM_{197}

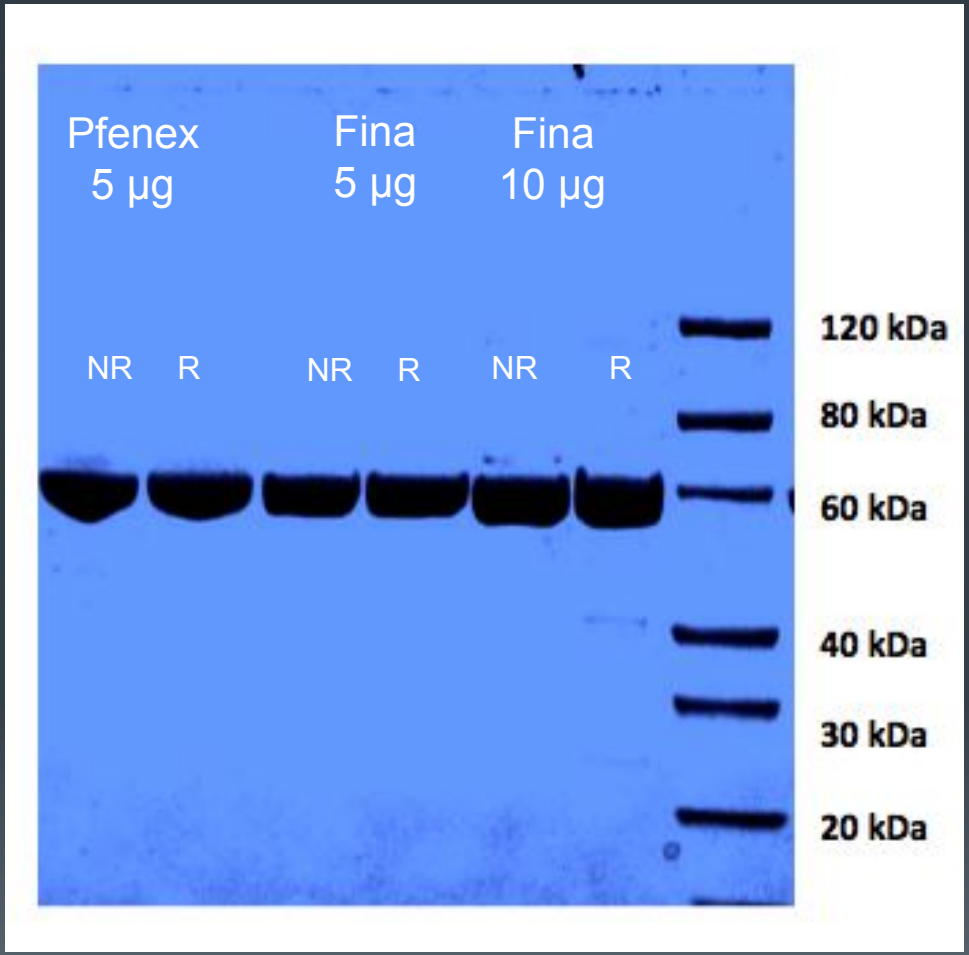
with support of PATH

- *E. coli* Protein Expression
  - Expression vector designed for intracellular *E.coli* expression
  - Expression strain optimization
  - Functional assay for proper folding
  - Expression conditions evaluation, expression yield and solubility optimization
  - Simple purification process

EcoCRM™:
Economical CRM_{197} from *E.coli*

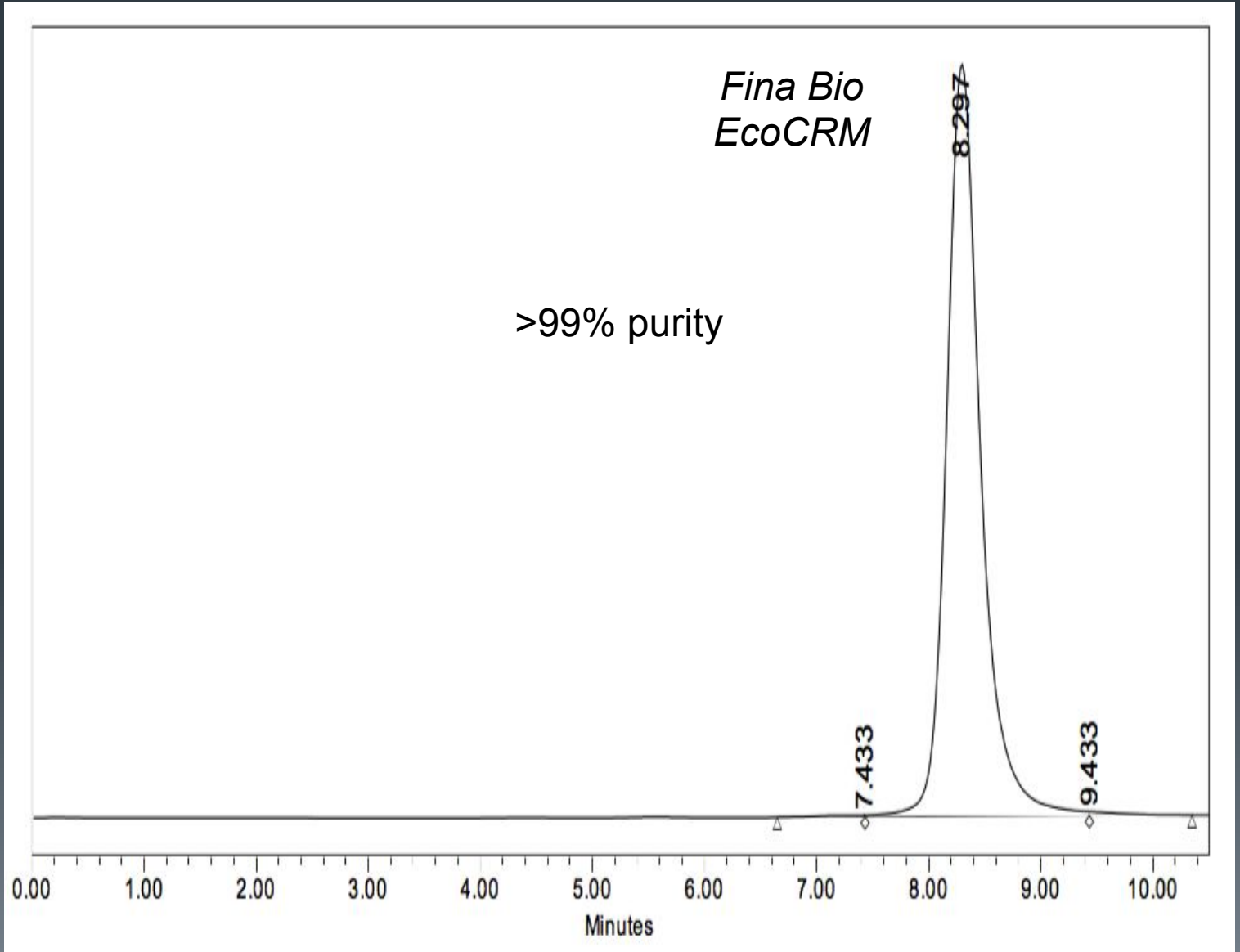
- Expressed as soluble proper folded protein
- Native sequence
- Simple low cost fermentation in well-accepted *E.coli* strain and very high expression levels
- Straightforward purification process
- Minimal “nicking”
- Excellent solubility properties

EcoCRM™ Characterization



Endotoxin: <5.0 EU/mg
Nucleic acid: not detectable
Minimal cleavage

EcoCRM™ SEC HPLC



Efficient Conjugation Chemistry

Improving yields

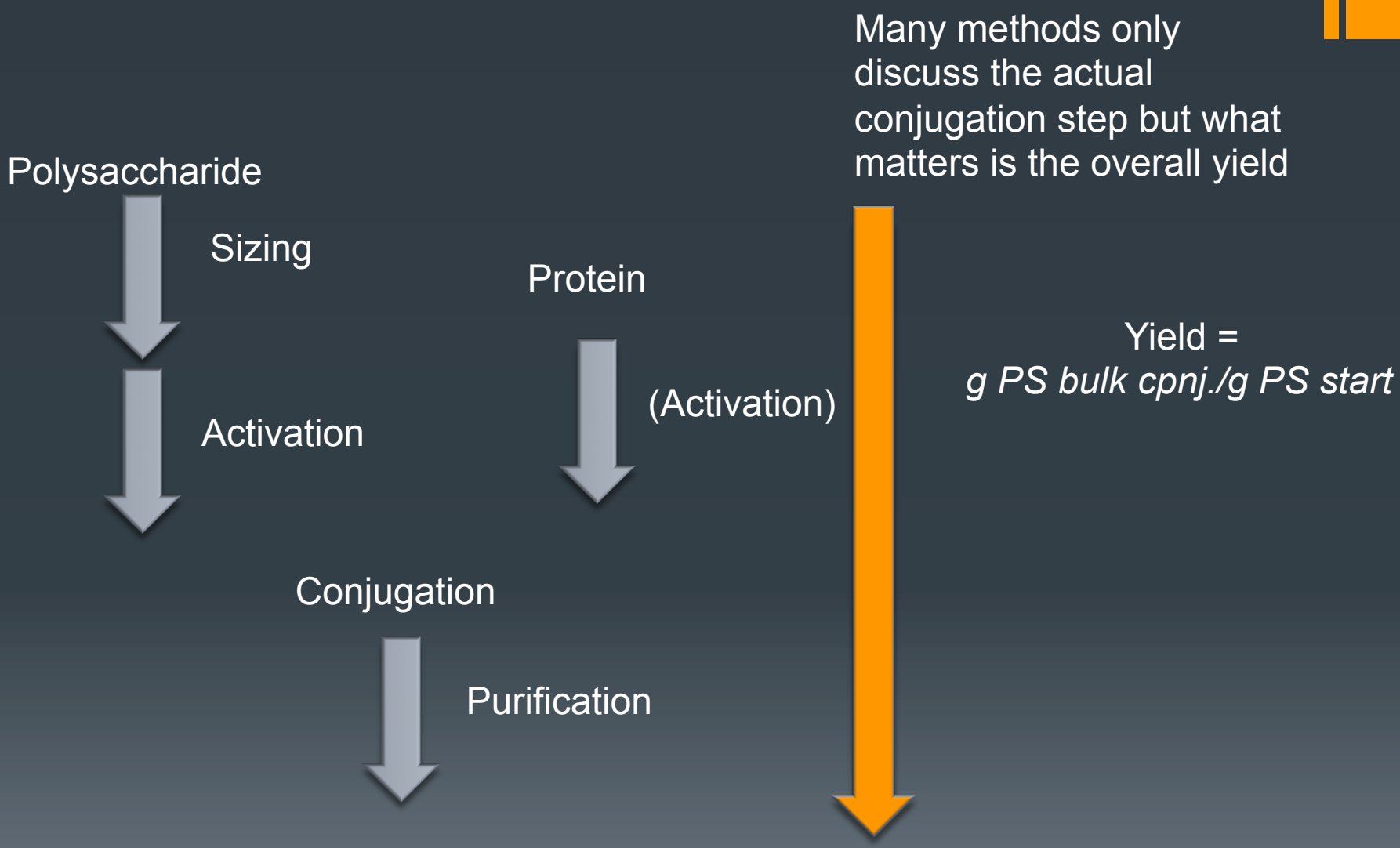
Efficient Conjugation Chemistry
CRM COGS vs Conjugation Efficiency

Current minimum cost estimate CRM ~$4000/g

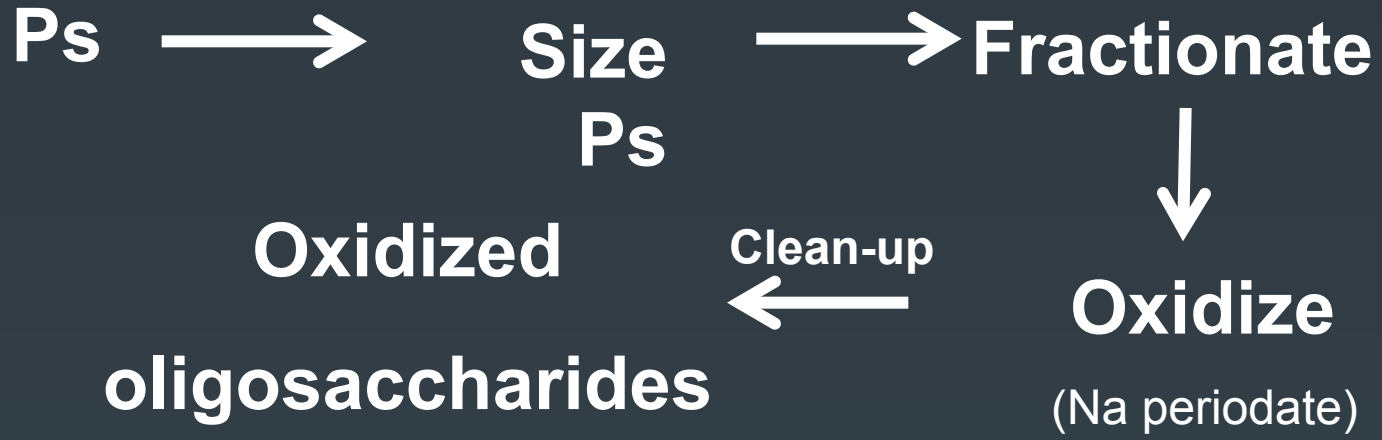
Cost per 50 µg conjugated CRM

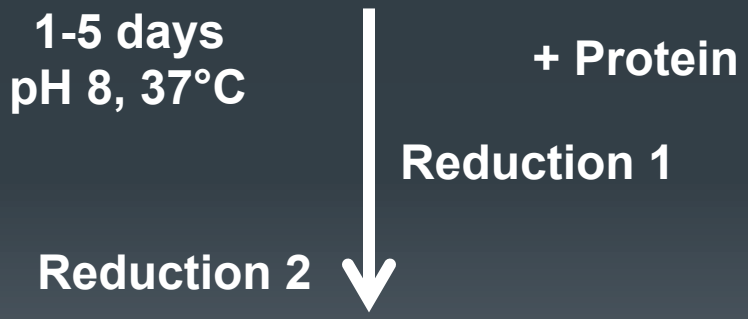
Conjugation Efficiency	$4000/g	$2000/g	$1000/g
100%	$0.20	$0.10	$0.05
50%	$0.40	$0.20	$0.10
25%	$0.80	$0.40	$0.20
15%	$1.33	$0.67	$0.33

Conjugation Overview



Reductive Amination (Prevnar)

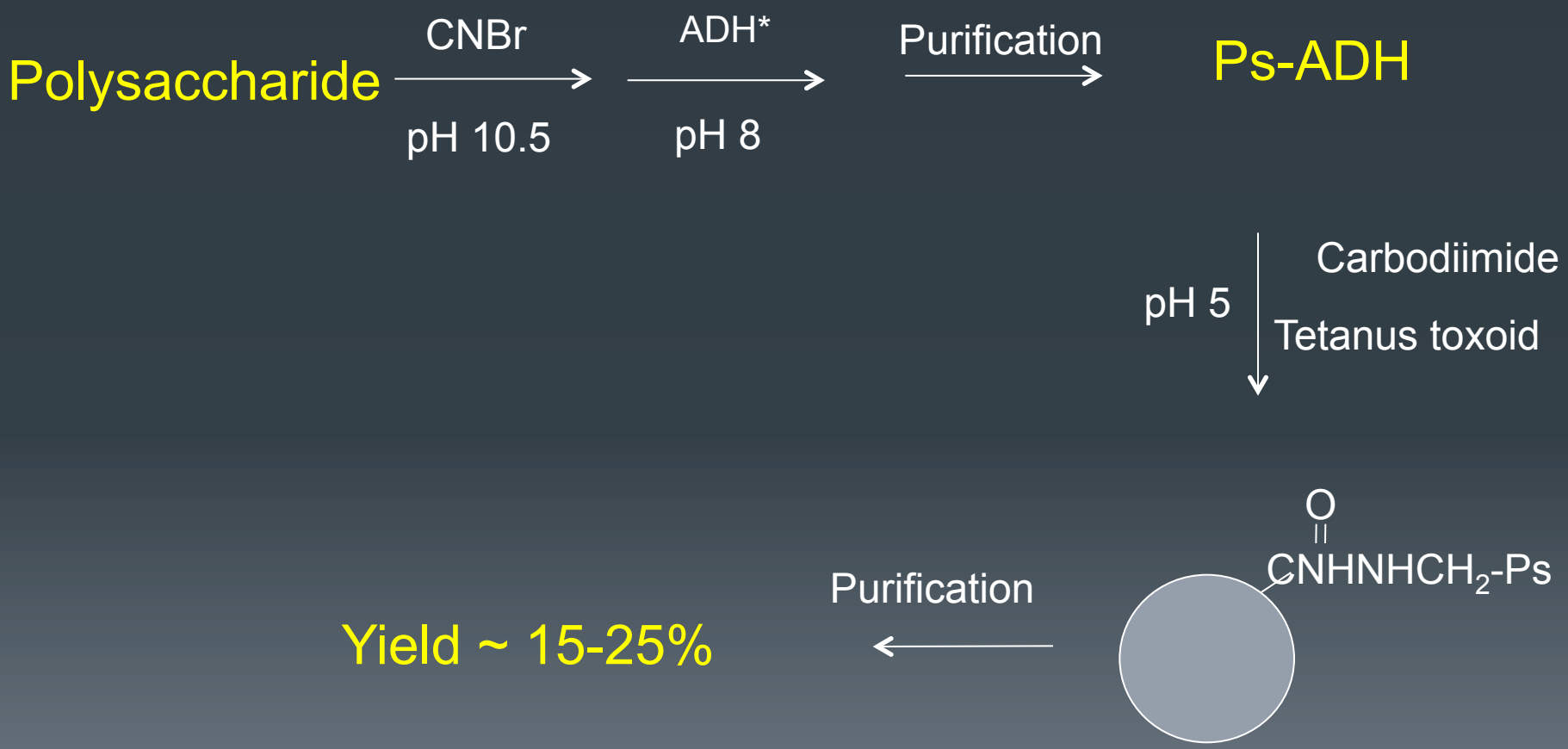




- *Many steps*
- *Slow*
- *Low yield (10-25%)*

Purify conjugate

Cyanogen Bromide
(Hib "Robbins' method")



CDAP Conjugation
(Synflorix)

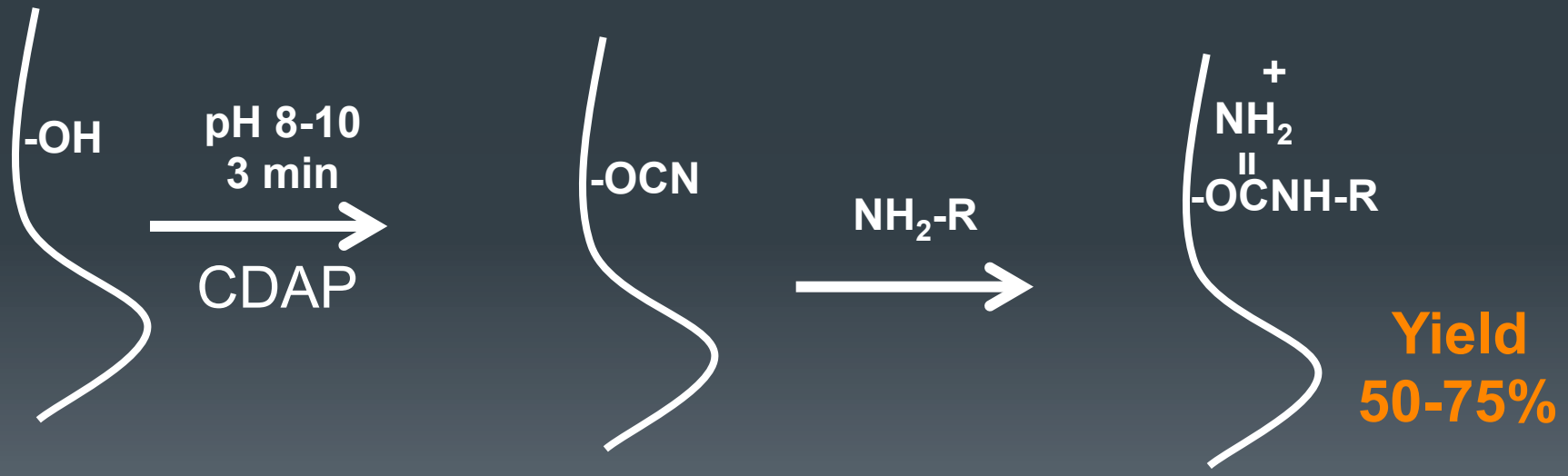
Short Reaction time

Lower pH

Direct conjugation

Rapid

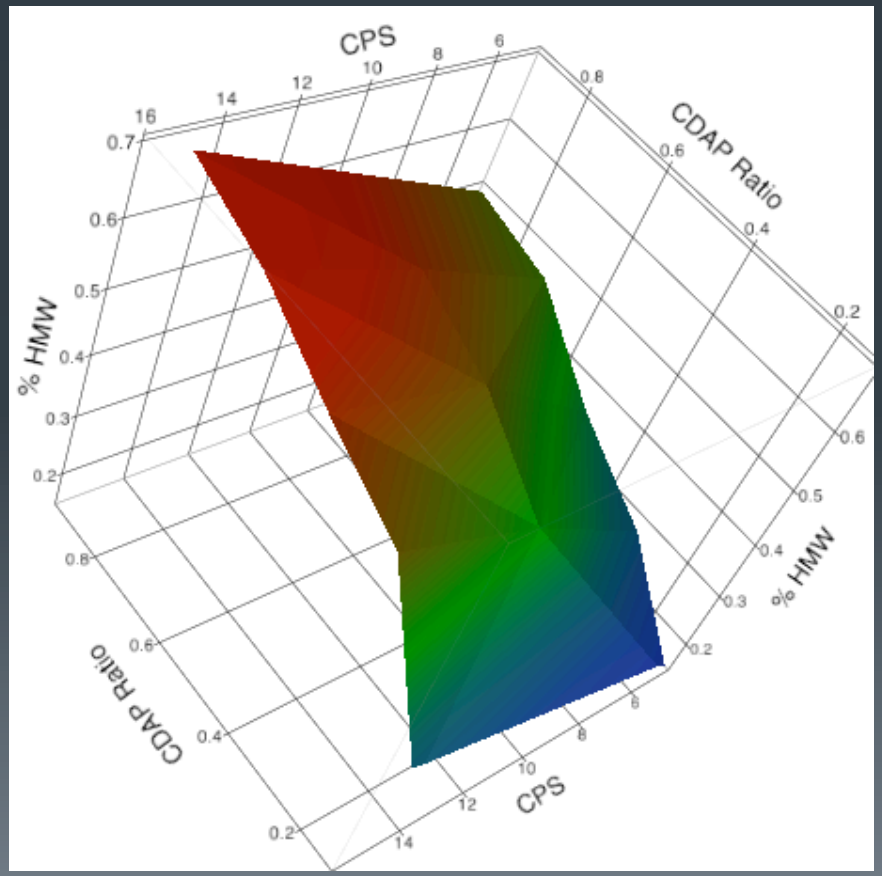
Stable isourea



Yield 50-75%

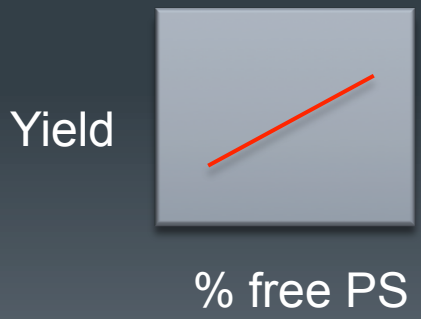
Design of Experiment (DOE)

- *A statistical method to model a process*
- *Many fewer experiments than "one factor at a time"*
- *Allows for modeling interactions*
- *Useful to determine critical parameters*
- *Critical for "Quality by Design"*



Conjugate Purification

- Conjugate vaccine synthesis should be viewed as a complete process
- Overall process yield is more important compared to yield at each step
- Use appropriate MW polysaccharide to facilitate purification
  - Size exclusion chromatography
  - Tangential flow filtration



QC Testing of Pneumococcal Conjugate Vaccine

Polysaccharide

1. Identity
2. Polysaccharide composition
3. Moisture content
4. Protein impurity
5. Nucleic acid impurity
6. Pyrogen content
7. Molecular size distribution

Activated saccharide

1. Extent of activation
2. Molecular size distribution

Conjugation

Carrier Protein

1. Identity
2. Purity
3. Toxicity
4. Extent of derivatisation (if appropriate) NR

Bulk Conjugate

1. Identity
2. Residual reagents
3. Saccharide:protein ratio & conjugation markers
4. Capping markers
5. Saccharide content NR
6. Conjugated v. free saccharide
7. Protein content
8. Molecular size distribution
9. Sterility
10. Specific toxicity of carrier (if appropriate)
11. Endotoxin content

Formulation

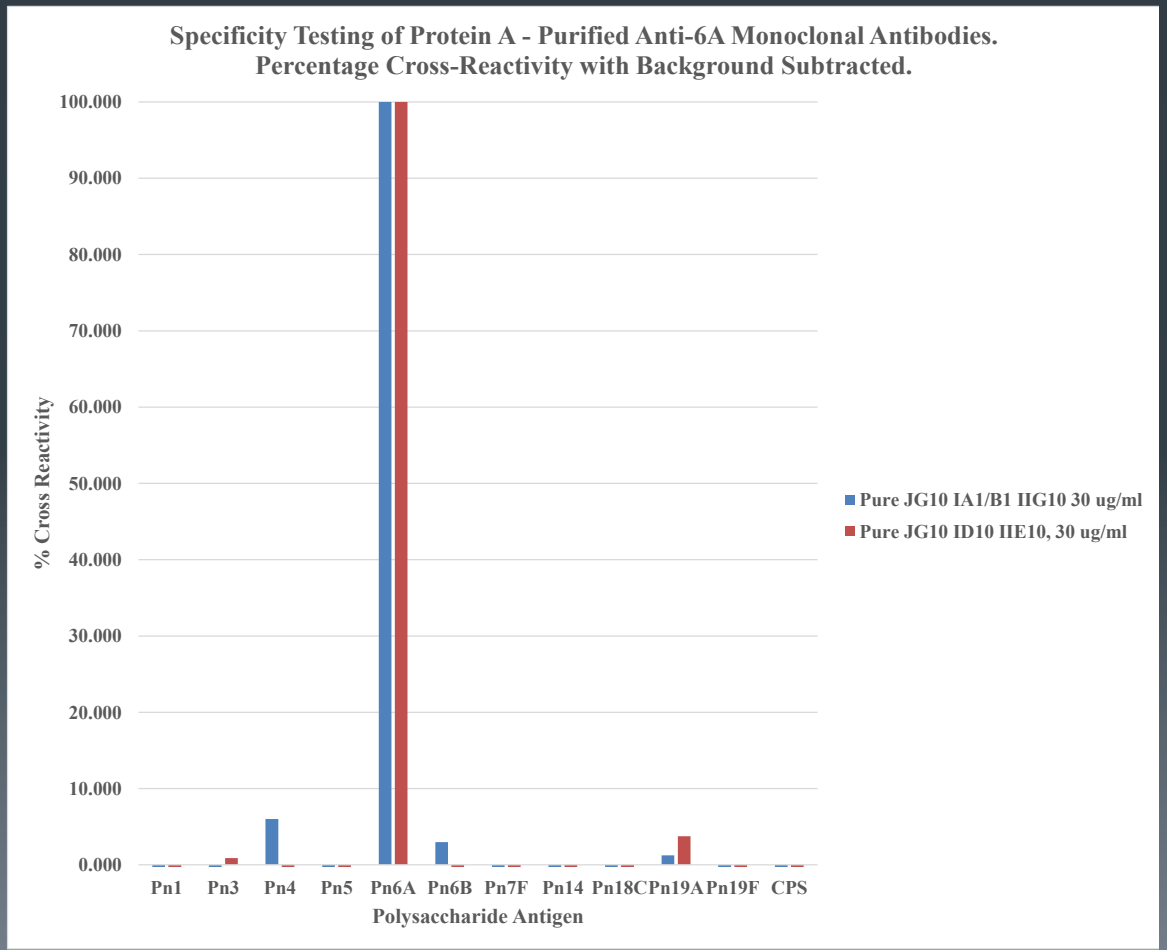
Final Vaccine

1. Identity
2. Sterility
3. Saccharide content (of each)
4. Residual moisture
5. Endotoxin content
6. Adjuvant content (if used)
7. Preservative content (if used)
8. General safety test
9. pH
10. Inspection

WHO Recommendations for the production and control of pneumococcal conjugate vaccines, ECBS, October 2003. Updated 2009

- Fina Biosolutions, Antibody Immunoassay Consultants and PATH
- Monoclonal antibodies against 13 Prevnar serotypes.
- Supply agreements to support clinical development

Monoclonal Antibodies

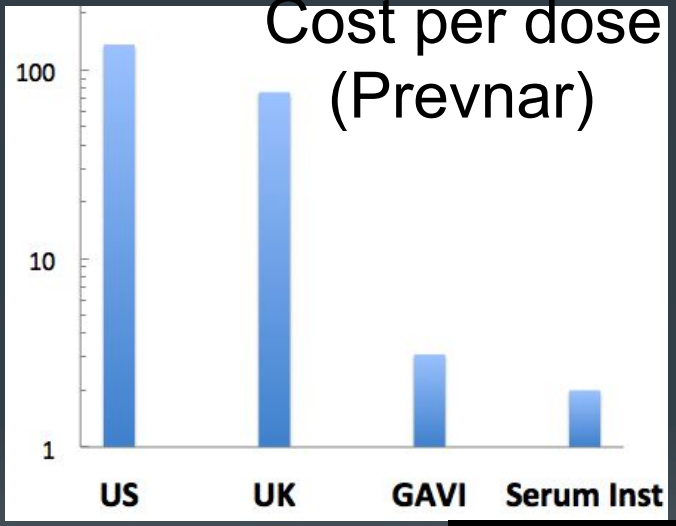


Prevnar 2015 Sales: $6.245 Billion

2015 sales (9 months)

US $2.773 Billion

Entire rest of the world $1.611 Billion







Towards Affordable Conjugate Vaccines Summary

- Appropriate business model
- Increase number of suppliers
- Affordable carrier protein
- Efficient conjugation chemistry

